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Urocortin 2 Protects Against Pacing-Induced Alternans via Phosphorylation of Phospholamban in Cardiac Myocytes from Normal and Failing Hearts

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Cardiac alternans is a high risk indicator for cardiac arrhythmias, stroke and sudden cardiac death. The cardioactive peptide Urocortin 2 (Ucn2) exhibits beneficial effects in normal and failing hearts, and elicits PKA-dependent positive inotropic and lusitropic effects in normal myocytes. Thus, we investigated if Ucn2 protects against pacing-induced alternans and elucidated the underlying mechanism.

Experiments were performed on single rabbit atrial and ventricular myocytes from normal and failing hearts. Chronic heart failure was induced by combined pressure and volume overload. Ca alternans was induced by incrementally increasing the pacing frequency until stable Ca alternans occurred at room temperature. Global Ca transients were measured with the fluorescent Ca indicators Fluo-4 or Indo-1 and monitored simultaneously with mechanical alternans (sarcomere length). In some experiments, cytosolic Ca alternans and intra-SR Ca alternans were simultaneously recorded with the Ca indicators Rhod-2 and Fluo-5N, respectively.

The average alternans ratio (AR = 1-(small-amplitude/large-amplitude)) in atrial myocytes was 0.79, and in normal and failing ventricular myocytes the ARs were 0.69 and 0.64, respectively. Ucn2 (100 nM) completely abolished Ca and mechanical alternans (within 2-3 min) in atrial and ventricular myocytes from normal and failing hearts. An increased sarcoplasmic reticulum (SR) Ca content, together with an enhanced SR Ca release flux, suggested that Ucn2 normalized alternans through effects on SR Ca ATPase (SERCA). Ucn2 increased significantly the level of cyclic adenosine monophosphate (cAMP) in normal cells (~12-fold), and enhanced phosphorylation of phospholamban (PLB) at Ser16 in normal myocytes (~10-fold) and to a lesser extent (~5-fold) in failing myocytes. These data demonstrate that Ucn2 rescues alternans presumably via increased SERCA activity in atrial and ventricular myocytes and thus protects normal and failing hearts from proarrhythmic alternans.

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NOS1AP Modulates Intracellular Ca^{2+} in Cardiac Myocytes and is Up-Regulated in Dystrophic Cardiomyopathy

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NOS1AP gene (nitric oxide synthase 1-adaptor protein) is strongly associated with abnormalities in the QT interval of the electrocardiogram and with sudden cardiac death. To determine the role of NOS1AP in the physiology of the cardiac myocyte, we assessed the impact of silencing NOS1AP, using siRNA, on $[Ca^{2+}]i$ transients in neonatal cardiomyocytes. In addition, we examined the co-localization of NOS1AP with cardiac ion channels, and finally, evaluated the expression of NOS1AP in a mouse model of dystrophic cardiomyopathy.

Using siRNA, NOS1AP levels were reduced to ~30% of the control levels ($p<0.05$). NOS1AP silencing in cardiac myocytes reduced significantly the amplitude of electrically evoked calcium transients ($p<0.05$) and the degree of S-nitrosylation of the cells ($p<0.05$). Using confocal microscopy, we evaluated NOS1AP subcellular location and interactions with other proteins by co-localization analysis. NOS1AP showed a high degree of co-localization with the L-type calcium channel and the inwardly rectifying potassium channel Kir3.1, a low degree of co-localization with the ryanodine receptor (RyR2) and alfa-sarcomeric actin and no co-localization with connexin 43, suggesting functionally relevant interactions with the ion channels that regulate the action potential duration.

Finally, using immunofluorescence and Western blotting, we observed that in mice with dystrophic cardiomyopathy, NOS1AP was significantly up-regulated ($p<0.05$).

These results suggest for a role of NOS1AP on cardiac arrhythmias, acting on the L-type calcium channel, and potassium channels, probably through S-nitrosylation.

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pGz Reverses Cardiac Dysfunction in Dystrophic Mice

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Duchenne muscular dystrophy (DMD) is caused by the absence of dystrophin expression and is one of the most common genetic diseases of children worldwide. Duchenne cardiomyopathy (D-CM) is observed in all patients suffering from DMD as the disease progresses. Despite knowing the cause of DMD and D-CM, there are no treatments which reverse its inevitable progression.

Periodic Acceleration (pGz) is a novel method that applies repetitive, sinusoidal, head-to-foot motion to a horizontally positioned body to induce increased pulsatile shear stress in fluid channels throughout the entire body. pGz increases the expression of eNOS, and nNOS and other endothelial derived proteins. Experiments were conducted in cardiomyocytes isolated from Wt and mdx mice (3, 6, 9 and 12-mo), treated or not with pGz (one hour for 16 consecutive days). Resting Ca^{2+} ($[Ca^{2+}]r$) and Na^{+} ($[Na^{+}]r$) were determined using Ca^{2+} and Na^{+} selective microelectrodes. pGz did not modify $[Ca^{2+}]r$ or $[Na^{+}]r$ in cardiomyocytes from Wt mice at any age (3, 6, 9 and 12-mo). In contrast, pGz restored $[Ca^{2+}]r$ and $[Na^{+}]r$ to Wt levels in cardiomyocytes from 3, 6 and 9-mo mdx mice and significantly reduced $[Ca^{2+}]r$ and $[Na^{+}]r$ in those from 12-mo old mdx mice. To assess the [NO]i Wt and mdx cardiomyocytes isolated from mice of 3, 6, 9 and 12-mo of age were loaded with 10μM DAF-FM DA (Molecular Probes, OR, USA). pGz did not significantly modify DAF fluorescence in Wt cardiomyocytes at any age. In contrast, pGz reduced DAF-Fluorescence in treated mdx cardiomyocytes at 3,6, 9 and 12 mo of age compared to untreated mdx cardiomyocytes of the same age. Our results suggest that pGz can be a drug free therapeutic approach to treat the cardiomyopathy observed in patients suffering from D-CM.

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A Markov-State Model for the Regulation of the Sarcoplasmic Reticulum Ca^{2+} ATPase by Phospholamban

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Compelling evidence suggests that reduced sarcoplasmic reticulum (SR) Ca^{2+} uptake via the SR Ca^{2+} ATPase (SERCA) is a major contributor to the development of Ca^{2+} signaling abnormalities in heart failure, and equally important as other known contributors to impaired Ca^{2+} handling.

Mathematical models of Ca^{2+} uptake via SERCA have been proposed, including recent 'state-model' descriptions that represent distinct catalytic states of the ATPase.

While these state models have answered important questions about the pump's dependence on Ca^{2+}, Mg^{2+}, and the free energy of ATP hydrolysis, to our knowledge state-based models that account for SERCA's molecular interaction with the endogenous phospholamban inhibitor are less well-developed.

We thus propose a SERCA Ca^{2+} uptake model that is based on a sequence of crystallographically-determined conformational states.

These distinct conformations have been shown to give rise to the cooperativity of cytosolic Ca^{2+} binding, and the kinetics of which are altered by phospholamban binding.

We apply the new SERCA model to a 3-dimensional model of Ca^{2+} signaling in a realistic, confocal-microscopy derived cardiac ventricular myocyte, with which we demonstrate the effects of altered Ca^{2+} cooperativity on the Ca^{2+} transient.

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Optimal Reticulated Coverage of the Sarcoplasmic Reticulum

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During every heartbeat, the Ca^{2+} that is released must be reabsorbed into the sarcoplasmic reticulum (SR) sufficiently rapidly to allow the heart to relax and refill. Taking an economic viewpoint, we hypothesized that the organism would optimally balance the need to absorb Ca^{2+} sufficiently rapidly and the metabolic cost of producing SR ATPases (SERCA2). To test this hypothesis we simulated the release and uptake of Ca^{2+} with different reticulation topologies and fractional covering. We defined a cost function that includes the cost of ATPase production, a penalty for not absorbing Ca^{2+} sufficiently rapidly, and a penalty for insufficient Ca^{2+} binding to contractile proteins. We found that the optimal SR fractional covering (where a value of 1 corresponds to